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X-ray structure reveals a new class of alkaline phosphatase

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Alkaline phosphatases (EC 3.1.3.1) hereafter referred to as APs, are metalloenzymes that hydrolyze the phosphate monoesters under alkaline pH conditions. APs are widely distributed in nature from prokaryotes to eukaryotes, and show substantial divergence with respect to their molecular weight, pH and temperature optima, thermal stability, metal requirement, specific activity and cellular location. The AP isolated from the bacterium *Sphingomonas.* sp. Strain BSAR-1 (SPAP), is a novel enzyme with properties very suited for use in molecular biology and bioremediation applications. Here we report the first three dimensional crystal structure of SPAP bound by an organic phosphate monoester. The structure is solved by MAD method using selenomethionine derivative. Native structure is refined to 1.95 Å resolution with R and R_{free} of 15.53 and 18.63 respectively. The structure shows that SPAP is the first AP in which: 1) the catalytic residue is threonine instead of serine and 2) third metal ion binding pocket near the active site observed in all APs so far is abolished in SPAP. When compared to AP from *E. coli* (ECAP) and other ECAP-like APs, the architecture of the active site is similar only to the extent that a bimetallic zinc core is coordinated by conserved His and Asp residues, while the combination of residues binding the substrate phosphoryl group is different. Individual residues of this combination are observed only separately in different members of the AP superfamily.

The conserved arginine residue in the active site of AP from *E. coli* (ECAP) is deleted in SPAP, and a lysine, an arginine and an aspargine residue recruited together for the first time into the active site, bind the substrate phosphoryl group in a manner not observed before in any other AP. The characteristic pair of hydrogen bonds between active site arginine and nonbridging posphoryl oxygens from the substrate is replaced by two non-coplanar hydrogen bonds contributed by Asn and Arg. This mode of interaction may be an alternate way of stabilizing the bi-pyramidal transition state in alkaline phosphatases. A positively charged lysine residue interacts directly with two nonbridging phosphoryl oxygens, and this could lead to enhanced catalysis. These and other structural features suggest that SPAP represents a new class of APs, additional members of which could be discovered in the future. However, the orientation of the phosphoryl moiety in the active site is consistent with the in-line displacement mechanism mediated by a trigonal-bipyramidal transition state proposed for ECAP-like APs. The structure is suggestive of independent evolutionary paths for SPAP and ECAP-like APs, and also of the likelihood that NPPs evolved from SPAP-like structure rather than from ECAP-like structure as suggested earlier. The structure can be used to identify residues that can be modified to engineer SPAP for molecular biology applications.

Keywords: X-ray structure, alkaline phosphatase, MAD

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Structural insights into the mechanism of drug activation by azoreductases

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Drug metabolism in the gut is an important but so far poorly understood part of the process of drug absorption into the body. For some time azoreductases have been known to be vital for the activation of azo prodrugs such as balsalazide in the gut. Azoreductases are NAD(P)H dependent flavoenzymes found in species ranging from bacteria to humans [1]. As well as being important for drug metabolism knockout studies in bacteria suggest azoreductases might be valid targets for novel antibiotics. Azoreductases are able to reduce a chemically diverse range of substrates including azo drugs, quinones, nitroaromatics and metal ions. The mechanism by which they reduce these varied substrates has remained elusive until recently. Three azoreductases from *P. aeruginosa* have been extensively characterized [1], [2]. Recent structural studies have focused on one of these enzymes (paAzoR1) and have significantly improved our understanding of their reaction mechanism. Structural data showing paAzoR1 bound to the inflammatory bowel disease prodrug balsalazide (PDB: 3LT5) have significantly altered our perception of how azoreduction occurs and provide direct evidence for the mechanism of quinone reduction [1]. The proposed quinone reduction mechanism has subsequently been supported by the structures of paAzoR1 bound to Coenzyme Q_1 and anthraquinone-2-sulphonate. Data from paAzoR1 bound to the topical antibiotic nitrofurazone, have provided evidence for the mechanism of nitroaromatic reduction by azoreductases and suggests there may be a conserved mechanism among structurally diverse nitroreductases [3].

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Keywords: azoreductase, mechanism, nitroaromatic

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First structural evidence for the order of preference of inorganic substrates by lactoperoxidase

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Lactoperoxidase (LPO) catalyzes the oxidation of halides and pseudohalides by hydrogen peroxide to produce a wide range of antimicrobial activity. LPO has been co-crystallized with a mixture containing equimolar concentrations of SCN^-, I^-, Br^- and Cl^-. The structure determination revealed the positions of four linearly arranged halides in the substrate-diffusion channel. The bound substrate ions are separated from each other by one or more water molecules. The heme iron is coordinated to His-351 $N^{\varepsilon 2}$ on the proximal side while it is coordinated to conserved water molecule W-1 on the distal heme side. W-1 is hydrogen bonded to Br^- ion which is followed by Cl^- ion with a hydrogen bonded water molecule W-5′ between them. Next to Cl^- ion is a hydrogen bonded water molecule W-7′ which is hydrogen bonded to W-8′ and N atom of SCN^- ion. W-8′ is hydrogen bonded to W-9′ which in turn is hydrogen bonded to I^- ion. I^- is also hydrogen bonded to Thr-425 N. On the other hand SCN^- interacts directly with Asn-230 and through water molecules with Ser-235 and Phe-254. According to the locations of four substrate anions in the channel with respect to the

conserved water molecule W-1, the order of preference for binding to lactoperoxidase appears to be as Br^- first, Cl^- second, SCN^- third and I^- the last. The positions of these anions in the substrate binding site are further defined in terms of subsites where Br^- is located in subsite 1, Cl^- in subsite 2, SCN^- in subsite 3 and I^- in subsite 4.

Keywords: antimicrobial, heme, oxidation

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Structure and mechanism of chloromomuconolactone dehalogenase from *Rhodococcus opacus* 1CP

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Chloroaromatic compounds are often very persistent environmental pollutants. Nevertheless numerous bacteria are able to metabolise these compounds and use them as the sole energy and carbon source. *Rhodococcus opacus* 1CP is able to degrade 3-chlorocatechol via a unique variant of the modified *ortho*-pathway. This pathway involves chloromuconolactone dehalogenase which dehalogenates the 5-chloromuconolactone to *cis*-dienelactone. The enzyme shows a high similarity to muconolactone isomerases, but is not able to catalyse the isomerisation reaction. In order to characterize the catalytic mechanism of this unusal dehalogenase, we crystallised the enzyme and subjected it to X-ray structural analysis. Datasets of up to 1.65 Å resolution were collected from two different crystal forms using synchrotron radiation. Cocrystallisation with substrate analogs yielded to crystals which are currently analysed for binding, to characterise the mechanism of dehalogenation.

Keywords: biotechnology, biocrystallography, enzyme

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L-*N*-carbamoylase structure suggests a striking case of protein evolution

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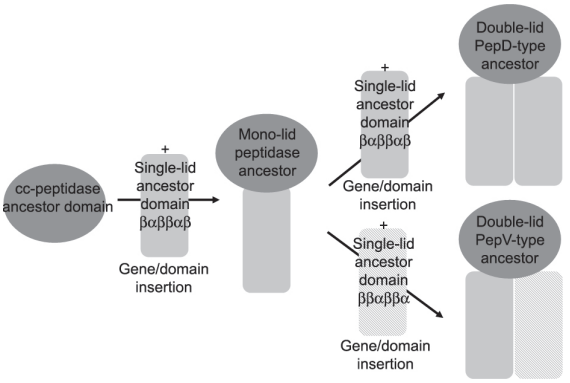
N-Carbamoyl-L-amino acid amidohydrolases (L-carbamoylases, E.C. 3.5.1.87) are important industrial enzymes used in the kinetic resolution of racemic mixtures of *N*-carbamoyl-amino acids due to their strict enantiospecificity [1]. This work reports the first L-carbamoylase structure belonging to *Geobacillus stearothermophilus* CECT43 (BsLcar) at 2.7 Å resolution showing the typical lung-shaped scaffold from the peptidase M20/M25/M40 family [2]. This common architecture is formed by two domains which for other homologs are known as catalytic and lid domains, respectively. Structural analysis of BsLcar and several members of the peptidase M20/M25/M40 family reveals several positionally conserved residues in this family, which can be hypothesized to have a critical role in substrate binding for the whole group of enzymes. An unexpected molecule bound in the catalytic cleft confirms large-scale rearrangements toward the lid domain for substrate hydrolysis, and allows us to infer the mechanism governing domain motion of L-carbamoylases. Comparative studies of the lid domain reveal a new "small molecule binding domain" (SMBD) belonging to the ACT-like superfamily. Based on BsLcar structure we propose an evolutionary pathway for all members of the peptidase M20/M25/M40 family through domain fusion and convergent/divergent evolution from common peptidase and SMBD ancestors.



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Crystal structures of cytochrome c peroxidase from *Pseudomonas stutzeri* in active and inactive forms.

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Bacterial di-heme Cytochrome *c* Peroxidase (CCP) is essential to maintain H_2O_2 below toxic levels by catalyzing its reduction to H_2O. Bacterial CCP consists of two heme domains, one harboring the electron transfer heme (E heme) and the other the peroxidatic heme (P heme). The crystal structure of the CCP from *Pseudomonas stutzeri* was obtained in two different redox states. The oxidized form (inactive) crystal structure was refined to 1.6 Å resolution[1]. In this structure the peroxidatic heme is coordinated to six ligands. The reduced form, in the active mixed valence state, was refined to 2.02 Å resolution and has a water molecule bound to the peroxidatic heme. These two structures have significant conformational differences in some regions, in particular around the P heme and the interface between the two domains. Previous studies indicate that CCP from *P. stutzeri* has a very high affinity for calcium [2]. This property has been addressed from a structural point of view. Structural data will also be obtained for the CCP from the same organism in the calcium free state in the oxidized form. These structures, along with the IN and OUT forms of *P. Nautica* [3] will help to obtain a better understanding of the electron transfer mechanism within di-heme CCP and also of the role of the calcium in its activation and mechanism.